EFFECTS OF INCORPORATING CHEMICAL LIGHT SOURCES IN CDC TRAPS: DIFFERENCES IN THE CAPTURE RATES OF NEOTROPICAL *CULEX, ANOPHELES* AND *URANOTAENIA* (DIPTERA: CULICIDAE)[1]

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Abstract.—Differential attraction of mosquitoes to chemical and incandescent light sources was compared using battery operated suction traps placed in a tropical lowland forest. Females of *Culex adamesi* Sirivanakarn, *Cx. amazonensis* (Lutz), *Cx. corniger* Theobald, *Cx. declarator* Dyar & Knab, *Anopheles mattogrossensis* Lutz & Neiva, *Aediomyia squamipennis* (Lynch), *Mansonia amazonensis* (Theobald), *Uranotaenia apicalis* Theobald, and *Ur. geometrica* Theobald were significantly attracted to chemically produced light. Light sources influenced the number of species attracted, the time (trap-nights) necessary to detect them, and the numbers of specimens collected per species.

Key Words.—Insecta, *Aediomyia*, light traps, mosquitoes, *Mansonia*, phototaxis

Data from light traps are subject to several systematic errors, or biases, which can complicate the interpretation of mosquito surveys. An important source of bias is the unequal phototactic responses of mosquitoes to different wavelengths and intensities of light. Because species do not respond alike, light trap collections may not approximate true species' abundances proportional to one another in nature, let alone their relative attraction to man (Huffaker & Back 1943). This can undermine the purpose of mosquito collections and affect survey time and labor costs.

Although phototactic responses present pitfalls in data interpretation, they do offer valuable opportunities to improve sampling regimes. Whether the intent is to capture many species of a fauna or many individuals of one species, judicious selection of an attractant can increase capture rates and shorten survey time.

The present study reintroduces a neglected method for quantifying losses and gains in efficiency produced by light trap attractants (Gaufin et al. 1956). In the process, some useful but seldom employed analysis techniques are examined for their value in pilot studies. Chemical light sticks are used as attractants, because a variety of them have recently become available commercially, and their portability may soon bring them into popular entomological use.

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[1] The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Navy or of the naval service at large.

METHODS AND MATERIALS

Testing Response to Light. — Data for testing responses were collected in a Latin square design of CDC light traps. Trap sites were located 20–50 m apart along the forest perimeter of the grounds of the Naval Hospital in Iquitos, Loreto Department, Peru. Treatments were five chemical light sticks (yellow, green, blue, white, and red; Cyalume®, American Cyanamid Company, One Cyanamid Plaza, Wayne, New Jersey 07470), an incandescent bulb (type CM49), and a control (a trap operating without light).

Treatments were assigned randomly to traps. Sticks (one per trap) were secured over the intake vents of CDC-style battery operated downdraft light traps (Model CDC-4; Hausherr's Machine Works, Old Freehold Road, Toms River, New Jersey 08753) from which the light bulbs were removed. Traps remained in place at sites, and sticks were switched each night, until the fauna of each site had been sampled once with each treatment.

The manufacturer's estimates of light duration were 12 hours (yellow, green, and red sticks) and 8 hours (white and blue). Traps were set at 1730 h and emptied at 0900; they ceased emitting blue and white light at 0130, but continued to emit red, green, and yellow until 0530, and incandescent light until 0900. Traps were operated on seven consecutive rainless nights in March 1989.

Female mosquitoes from light collections were identified to species and counted. Counts of common ($n > 40$) species were examined in separate Friedman tests, one test per species. Test hypotheses were, H_0: Treatments attracted equal numbers of females; H_1: At least one treatment attracted more females than at least one other. The Friedman test statistic, T2, was computed according to formulae cited by Conover (1980) to approximate the F distribution. This statistic was then used to calculate the minimum rank sum difference for multiple *a posteriori* comparisons among treatments, as detailed in Conover (1980).

Sampling Efficiency. — The March experiment on light response was replicated in several different sites in the same forest during June, October, and January, using only red, blue, green and yellow as treatments. Efficiency estimates were then derived by analyzing these replicates jointly with data from the March collection (25 sampling nights total). Analysis was based only on species that were present in all four months and had shown significant phototaxis in March testing.

Sampling efficiency was defined as the average rapidity with which species were discovered in the traps. This was inspected graphically by plotting changes in a statistic termed P_k by Gaufin et al. (1956). P_k measured the average probability of collecting a species not collected previously, by each treatment on each successive night. To compute P_k, each treatment was tabulated to reflect a distribution of the number of nights that resulted in capture of each mosquito species (nights/species/treatment). Coefficients $a_{i,k}$ were determined, representing the probability of a species occurring on the k-th night but none previously, given that it occurred in k nights out of $i = 1$ to $n = 25$ nights. These coefficients were multiplied by the probability of the species being found in only k nights out of n; the result was summed across all remaining k. Formally,

$$a_{i,k} = \frac{i \cdot C_{n-k+1,i}}{(n - k + 1) \cdot C_{n,i}},$$

and

$$P_k = \sum_{i=1}^{n-k+1} a_{i,k} \cdot (S_i/S),$$

where S_i = the number of different species appearing in i out of n samples, and S = the number of species in n. Program source code for these computations is available from the senior author. Computation and rationale has been discussed in detail by Gaufin et al. (1956), who provide a worked example based on a survey of aquatic benthos.

RESULTS

A total of 5749 females was captured by traps during the seven nights of collection in March. More than 36 species were represented, of which 11 were common enough to include in Friedman tests: *Anopheles mattogrossensis* Lutz & Neiva ($n = 198$), *Aediomyia squamipennis* (Lynch) ($n = 195$), *Culex adamesi* Sirivanakarn ($n = 1497$), *Cx. amazonensis* (Lutz) ($n = 271$), *Cx. corniger* Theobald ($n = 93$), *Cx. declarator* Dyar & Knab ($n = 1137$), *Mansonia amazonensis* (Theobald) ($n = 47$), *Ma. indubitans* Dyar & Shannon ($n = 68$), *Coquillettidia venezuelensis* (Theobald) ($n = 43$), *Uranotaenia apicalis* Theobald ($n = 54$), and *Ur. geometrica* Theobald ($n = 72$).

Tests on *Ma. indubitans* and *Cq. venezuelensis* revealed no significant differences between control traps and traps incorporating any of the light sources. Captures of the remaining nine species were significantly ($P < 0.05$) higher in lighted traps, with important differences depending on the light employed (Fig. 1).

Red, green, and yellow sticks were not equally attractive to most species. *Anopheles mattogrossensis* and *Ur. apicalis* were more attracted to yellow than to green ($P < 0.05$). Red was unattractive (i.e., indistinguishable from controls) to several species that were attracted ($P < 0.05$) by both yellow and green, including: *Cx. adamesi, Cx. amazonensis, Cx. corniger, Cx. declarator, An. mattogrossensis, Ad. squamipennis,* and *Ma. amazonensis. Uranotaenia apicalis* and *Ur. geometrica* were attracted to yellow, but not to green or red. These capture differences cannot be ascribed to duration of light emission, inasmuch as red, green, and yellow sticks each emitted light for 12 hours per night.

Similarly, blue and white sticks each emitted light for eight h, but *Cx. adamesi* was more attracted by white than by blue.

Incandescent light was the most attractive source for *Ur. apicalis* and *Ur. geometrica* ($P < 0.05$). *Cx. amazonensis* could perceive incandescent light ($P < 0.05$), but was more attracted by chemical light sticks ($P < 0.05$).

Six of the species tested in March (*Cx. adamesi, Cx. amazonensis, Cx. corniger, Cx. declarator, Ad. squamipennis* and *Ma. amazonensis*) were also present in June, October, and January, although in much reduced numbers. The sampling efficiency of light sticks was compared with respect to these six species. In 25 nights of sampling divided among the four months, all six species were detected by yellow sticks in an average of 11 days, by green in 13 days, by blue in 15 days, and by red in 25 days.

Sampling reward (the number of newly detected species) was greatest during the first two nights of trapping with light, indicated by increased slope near the

Aediomyia squamipennis

13	Red
15.5	Control
28	White
30	Blue
35	Incan.
36	Green
38.5	Yellow

Anopheles mattogrossensis

12	Control
22	Red
23.5	White
25.5	Green
33	Blue
37	Incan.
43	Yellow

Culex adamesi

8.5	Control
19	Red
25.5	Blue
30.5	White
37.5	Yellow
37.5	Green
37.5	Incan.

Culex amazonensis

12.5	Control
18.5	Red
27	Incan.
32.5	Blue
32.5	Green
33.5	Yellow
39.5	White

Culex corniger

11.5	Control
20	Blue
21	Red
25	Yellow
29	White
29.5	Green
32	Incan.

Culex declarator

9	Control
14	Red
23	White
28	Blue
36.5	Yellow
38.5	Green
47	Incan.

Mansonia amazonensis

17.5	Control
19.5	Red
25.5	White
26.5	Green
33.5	Incan.
35.5	Blue
38	Yellow

Uranotaenia apicalis

18.5	Control
20.5	White
22.5	Green
25.5	Red
28.5	Blue
33.5	Yellow
47	Incan.

Uranotaenia geometrica

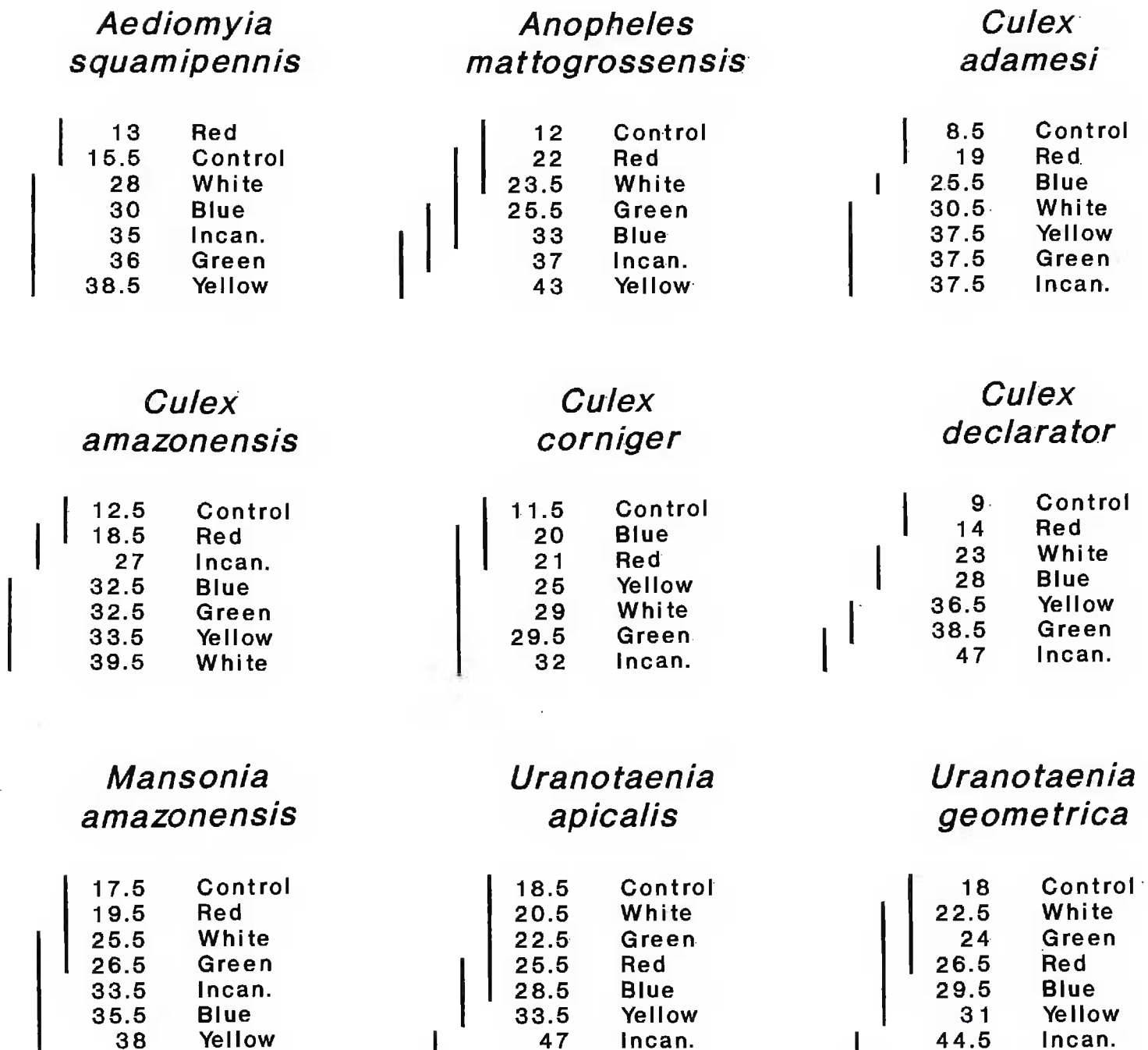| 18 | Control |
22.5	White
24	Green
26.5	Red
29.5	Blue
31	Yellow
44.5	Incan.

Figure 1. Relative attraction of female mosquitoes to chemical and incandescent light sources, ordered by rank sums of the Friedman test. Order is from least (top) to greatest (bottom) attraction. Sums not subtended by the same vertical line differ at $P < 0.05$.

origin of P_k curves (Fig. 2). For example, yellow detected four species in the first two nights of sampling, but took nine additional nights to detect all six species. Blue detected three species in the first two nights, and the remaining three species 13 nights later.

DISCUSSION

A Latin square arrangement is useful in removing two extraneous sources of variation from a desired comparison of treatments (Damon & Harvey 1987). This ability can be particularly effective in controlling the effects of time and place in a pilot study. Both effects are very real in mosquito surveys, due to the habitat preferences of mosquito species, and their fluctuating population sizes over time (Jones et al. 1991, Williams 1951). Actually, three unwanted sources of variation (location, time, and trap effects) commonly occur in mosquito surveys, but location and trap effects are pooled if traps are not moved while sampling. By leaving traps in the same collection stations during our experiments, trap variation
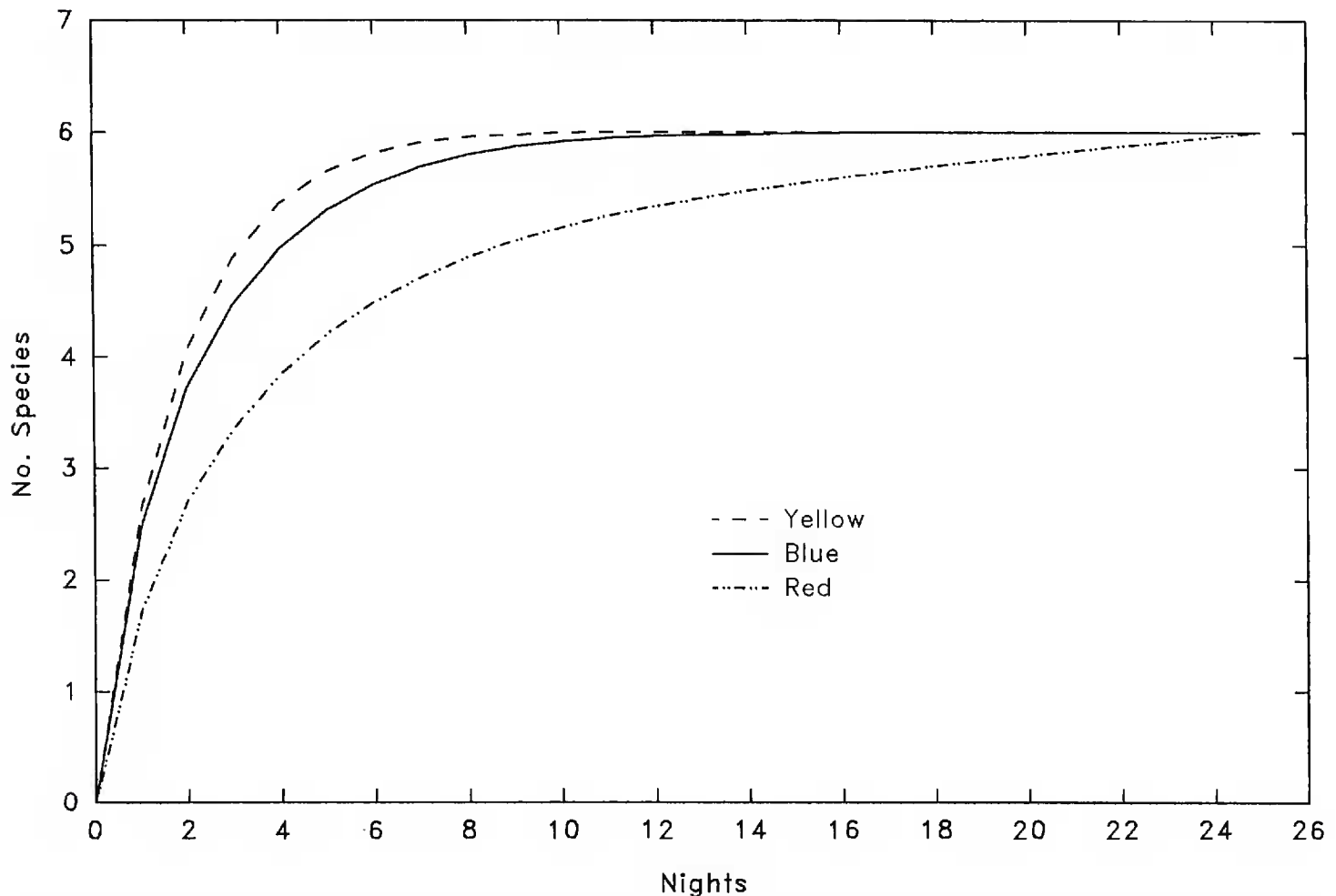
Figure 2. Average time required to detect six mosquito species at light sticks in CDC traps placed near Iquitos, Peru. The curve for green light sticks (not shown) lies very close to yellow.

(motor speed, bag resistance to air flow, etc.) was collapsed onto the location effects (shrubbery, wind, and so forth), and blocked.

An additional use of the Latin square is to subject data to a powerful non-parametric test of significance used for complete block designs, the Friedman test (Friedman 1940). This relatively old test is particularly suited to non-normal sampling distributions such as counts of insects in traps. The recent development of *a posteriori* error rates for it enhances its usefulness. Although the test is commonly used by ecologists, it is rarely employed in insect surveys. Entomologists have instead favored incompletely blocked designs (Belton & Pucat 1967, Holbrook & Bobian 1989, Rowley & Jorgensen 1967, Service & Highton 1980, Service et al. 1983, Slaff et al. 1983, Vavra et al. 1974), or transformed data and analyses based on assumed normality and homoscedasticity (Kline et al. 1991, Williams 1951, Williams et al. 1955). An exception is Anderson & Linhares (1989) in which the Friedman test was used to demonstrate the attractiveness of combined CO_2 and ultraviolet lures for *Culicoides variipennis* (Coquillett).

Friedman *a posteriori* contrasts (Fig. 1) indicate how to survey particular species in the Iquitos study site. For example, *Cx. declarator* and the *Uranotaenia* species should be sampled with an incandescent bulb instead of light sticks. *Cx. adamesi* is attracted to white, yellow, and green sticks, and to incandescent light, but should not be sampled with blue sticks. *Cx. amazonensis* should not be sampled with incandescent bulbs. *An. mattogrossensis* should not be sampled with green or white sticks. Red should not be used for any of the 11 species tested.

The strong performance of incandescent light in most tests may owe to the fact

that incandescent bulbs emit light longer than light sticks. Long duration of light emission is an advantageous quality that extends specimen collection from evening well into the following morning, thereby increasing trap exposure to crepuscular species. Nevertheless, the decision to use incandescent light depends on which species are of interest. For example, incandescent light lasting 15.5 h was definitely less productive in collecting *Cx. amazonensis* than were white sticks that last eight h (Fig. 1).

Because the Friedman test checks the equality of treatments, it is sensitive to the effects of mosquito repellency as well as attraction (positive and negative phototaxis). However, a control can be used to distinguish the two effects. Thus, there was no evidence of mosquito repellency by red or other treatments in the March survey, because the experimental control was never significantly ($P < 0.05$) more productive than any treatment in *a posteriori* comparisons. For the same reason, we cannot conclude that *Ma. indubitans* and *Cq. venezuelensis* were either repelled or attracted by light of any kind.

The generally poor performance of red sticks is noteworthy, as is the failure to capture *Ma. indubitans* and *Cq. venezuelensis* at light. Both observations are important from the practical standpoint of sampling this local fauna. However, we stress that these results should not be generalized to faunas composed of other species, nor to surveys of the same species in other localities. Pilot studies should always be conducted in the locale of interest, before conclusions are drawn.

Sampling efficiency is broadly defined as the cost necessary to obtain an estimate of a desired precision (Freese 1962). The cost can be stated in various currencies to serve specific purposes (Castleberry et al. 1989, Wilkinson & Gregson 1985, Zimmerman & Garris 1985). For mosquito surveys, which incur costs related to the nightly labor of servicing traps, it is intuitively meaningful to express efficiency as the number of trap nights needed to detect a given number of species. Viewed in these terms, the most efficient attractant is that which captures more species in less time than other attractants. It represents the best compromise for sampling a local fauna.

We, therefore, compared the average rapidity with which certain important species were recovered in traps, by a method that translated mosquito capture rates into time cost. It estimated the proportion of the species captured in a large number of nights that would have been detected, on the average, in a smaller number of nights. Under conditions prevailing in the study site during March, June, October, and January, yellow sticks were more efficient than red, blue or green in surveying a fauna composed of *Cx. corniger, Cx. adamesi, Cx. declarator, Cx. amazonensis, Ad. squamipennis,* and *Ma. amazonensis.* The amount of survey time saved by use of yellow to detect all six species ranged from two days (compared to green) to 14 days (compared to red).

Some practical generalizations deserve emphasis in conclusion. First, the advantage in choosing an efficient mosquito attractant is realized in a few initial evenings of use. Most of the species that can be detected are caught rather quickly, in about two nights. Second, chemical light sticks can be more productive than incandescent bulbs in collecting certain species. Finally, the amount of time needed to detect a given number of mosquito species depends upon which light stick is employed.

Literature Cited

Anderson, J. R. & A. X. Linhares. 1989. Comparison of several different trapping methods for *Culicoides variipennis* (Diptera: Ceratopogonidae). J. Am. Mosq. Control Assoc., 5: 325–334.

Belton, P. & A. Pucat. 1967. A comparison of different lights in traps for *Culicoides* (Diptera: Ceratopogonidae). Can. Entomol., 99: 267–272.

Castleberry, D. T., J. J. Cech Jr. & A. B. Kristensen. 1989. Evaluation of the effect of varying mosquito emergence on the efficiency of emergence traps over enclosed environments. J. Am. Mosq. Control Assoc., 5: 104–105.

Conover, W. J. 1980. Practical nonparametric statistics (2nd ed.). John Wiley & Sons, New York, New York.

Damon, R. A. & W. R. Harvey. 1987. Experimental design, ANOVA, and regression. Harper & Row, New York.

Freese, F. 1962. Elementary forest sampling. U.S. Dep. Agric. Handb.

Friedman, M. 1940. A comparison of alternative tests of significance for the problem of m rankings. Ann. Math. Statist., 11: 86–92.

Gaufin, A. R., E. K. Harris & H. J. Walter. 1956. A statistical evaluation of stream bottom sampling data obtained from three standard samplers. Ecology, 37: 643–648.

Holbrook, F. R. & R. J. Bobian. 1989. Comparisons of light traps for monitoring adult *Culicoides variipennis* (Diptera: Ceratopogonidae). J. Am. Mosq. Control Assoc., 5: 558–562.

Huffaker, C. B. & R. C. Back. 1943. A study of methods of sampling mosquito populations. J. Econ. Entomol., 36: 561–569.

Jones, R. E., P. Barker-Hudson & B. H. Kay. 1991. Comparison of dry ice baited light traps with human bait collections for surveillance of mosquitoes in Northern Queensland, Australia. J. Am. Mosq. Control Assoc., 7: 387–394.

Kline, D. L., D. A. Dame & M. V. Meisch. 1991. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas. J. Am. Mosq. Control Assoc., 7: 165–169.

Rowley, W. A. & N. M. Jorgensen. 1967. Relative effectiveness of three types of light traps in collecting adult *Culicoides*. J. Econ. Entomol., 60: 1478–1479.

Service, M. W. & R. B. Highton. 1980. A chemical light trap for mosquitoes and other biting insects. J. Med. Entomol., 17: 183–185.

Service, M. W., S. Sulaiman & R. Esena. 1983. A chemical aquatic light trap for mosquito larvae (Diptera: Culicidae). J. Med. Entomol., 20: 659–663.

Slaff, M., W. J. Crans & L. J. McCuiston. 1983. A comparison of three mosquito sampling techniques in northwestern New Jersey. Mosq. News, 43: 287–290.

Vavra, R. W., R. R. Carestia, R. L. Frommer & E. J. Gerberg. 1974. Field evaluation of alternative light sources as mosquito attractants in the Panama Canal Zone. Mosq. News, 34: 382–384.

Wilkinson, P. R. & J. D. Gregson. 1985. Comparisons of sampling methods for recording the numbers of Rocky Mountain wood ticks (*Dermacentor andersoni*) on cattle and range vegetation during control experiments. Acarologia, 26: 131–139.

Williams, C. B. 1951. Comparing the efficiency of insect traps. Bull. Entomol. Res., 42: 513–517.

Williams, C. B., R. A. French & M. M. Hosni. 1955. A second experiment on testing the relative efficiency of insect traps. Bull. Entomol. Res., 46: 193–204.

Zimmerman, R. H. & G. I. Garris. 1985. Sampling efficiency of three dragging techniques for the collection of nonparasitic *Boophilus microplus* (Acari: Ixodidae) larvae in Puerto Rico. J. Econ. Entomol., 78: 627–631.